

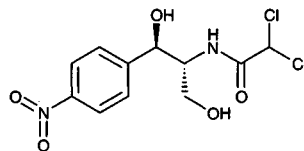
# Chloramphenicol

**Molecular formula:** C<sub>11</sub>H<sub>12</sub>Cl<sub>2</sub>N<sub>2</sub>O<sub>5</sub>

**Molecular weight:** 323.13

**CAS Registry No.:** 56-75-7

**Merck Index:** 2120



## SAMPLE

**Matrix:** blood

**Sample preparation:** Mix serum with an equal volume of 250 µg/mL 4'-nitroacetanilide in MeCN:MeOH 90:10, mix, let stand at room temperature for 10 min, mix, centrifuge at 12800 g for 2 min, inject a 25 µL aliquot of the supernatant.

## HPLC VARIABLES

**Guard column:** RCSS Guard-Pak (Waters)

**Column:** 100 × 8 C18 Radial Pak (Waters)

**Mobile phase:** MeOH:0.75% acetic acid 30:70, pH adjusted to 5.5 with triethylamine

**Flow rate:** 3

**Injection volume:** 25

**Detector:** UV 254, UV 280

## CHROMATOGRAM

**Retention time:** 9.5

**Internal standard:** 4'-nitroacetanilide (12.4)

**Limit of detection:** 2 µg/mL

## OTHER SUBSTANCES

**Extracted:** cefamandole, cefazolin, cefotaxime, cefoxitin, cephalirin

**Simultaneous:** acetaminophen, N-acetylprocainamide, cefaclor, cephalixin, cephalothin, cimetidine, miconazole, moxalactam, procainamide, sulfamethoxazole, theophylline, tobramycin, vancomycin

## KEY WORDS

serum

## REFERENCE

Danzer, L.A. Liquid-chromatographic determination of cephalosporins and chloramphenicol in serum, *Clin. Chem.*, **1983**, 29, 856–858.

## SAMPLE

**Matrix:** blood

**Sample preparation:** 500 µL Serum + 1 mL 1 M pH 3.0 sodium acetate + 5 mL diethyl ether, shake for 20 min, centrifuge at 1200 g. Remove the organic layer and evaporate it to dryness at 40° under a stream of nitrogen. Reconstitute the residue in 100 µL water, inject a 50 µL aliquot.

## HPLC VARIABLES

**Column:** 300 × 3.9 10 µm µBondapak C18

**Mobile phase:** MeCN:10 mM potassium acetate buffer 20:80, pH 6.5

**Flow rate:** 1.6

**Injection volume:** 50

**Detector:** UV 215

## CHROMATOGRAM

**Retention time:** 5.10

**Internal standard:** chloramphenicol

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#### OTHER SUBSTANCES

**Simultaneous:** penicillin V

**Noninterfering:** amoxicillin, ampicillin, tetracycline, doxycycline, cephalixin, amikacin, sisomicin, netilmicin, tobramycin, gentamicin, phenemal, phenacetin, ethosuximide, primidone, phenytoin, amiloride, hydrochlorothiazide

**Interfering:** penicillin G procaine, cloxacillin

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#### KEY WORDS

serum; chloramphenicol is IS

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#### REFERENCE

Lindberg, R.L.; Huupponen, R.K.; Huovinen, P. Rapid high-pressure liquid chromatographic method for analysis of phenoxymethylpenicillin in human serum, *Antimicrob. Agents Chemother.*, **1984**, 26, 300-302.

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#### SAMPLE

**Matrix:** blood

**Sample preparation:** 100  $\mu$ L Serum + 100  $\mu$ L buffer + 1.5 mL IS in 5% isopropanol in chloroform, vortex for 30 s, centrifuge. Remove the organic layer and evaporate it to dryness under a stream of air at room temperature, reconstitute the residue in 100  $\mu$ L mobile phase, inject a 6-10  $\mu$ L aliquot. (Buffer was 13.6 g  $\text{KH}_2\text{PO}_4$  in 90 mL water, pH adjusted to 6.8 with about 3 mL 10 M NaOH, made up to 100 mL.)

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#### HPLC VARIABLES

**Guard column:** 20  $\times$  4.6 Supelguard LC-1 (Supelco)

**Column:** 250  $\times$  4.6 5  $\mu$ m Supelcosil LC-1 (Supelco)

**Mobile phase:** MeOH:MeCN:buffer 17.5:17.5:65 (Buffer was 2.72 g  $\text{KH}_2\text{PO}_4$  in 1.9 L water, pH adjusted to 6.3 with about 2 mL 1 M NaOH, made up to 2 L.)

**Flow rate:** 2

**Injection volume:** 6-10

**Detector:** UV 273

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#### CHROMATOGRAM

**Retention time:** 3.85

**Internal standard:** 3-isobutyl-1-methylxanthine (3.15)

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#### OTHER SUBSTANCES

**Extracted:** acetaminophen, amobarbital, barbital, caffeine, carbamazepine, ethosuximide, mephobarbital, methsuximide, pentobarbital, phenobarbital, phenytoin, primidone, secobarbital, theophylline, thiopental

**Also analyzed:** acetanilide, N-acetylcysteine, N-acetylprocainamide, ampicillin, aspirin, butabarbital, butalbital, chlorpropamide, cimetidine, codeine, cyheptamide, diazoxide, diflunisal, diphyllyne, disopyramide, ethchlorvynol, gentisic acid, glutethimide, heptabarbital, hexobarbital, ibuprofen, indomethacin, ketoprofen, mefenamic acid, mephentoin, methaqualone, methsuximide, methyl salicylate, methypylon, morphine, naproxen, nirvanol, oxphenylbutazone, phensuximide, phenylbutazone, procainamide, salicylamide, salicylic acid, sulfamethoxazole, sulindac, tolmetin, trimethoprim, vancomycin

**Noninterfering:** amikacin, gentamicin, meprobamate, netilmicin, quinidine, tetracycline, tobramycin, valproic acid

**Interfering:** phenacetin

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#### KEY WORDS

serum

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**REFERENCE**

Meatherall,R.; Ford,D. Isocratic liquid chromatographic determination of theophylline, acetaminophen, chloramphenicol, caffeine, anticonvulsants, and barbiturates in serum, *Ther.Drug Monit.*, **1988**, *10*, 101-115.

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**SAMPLE**

**Matrix:** blood

**Sample preparation:** Prepare an SPE cartridge by plugging the end of a 1 mL disposable pipette tip with glass wool and adding about 100 mg Chromosorb P/NAW. Add 50  $\mu$ L plasma then 50  $\mu$ L 10  $\mu$ g/mL tolylphenobarbital in 200 mM HCl to the SPE cartridge, let stand for 2 min, elute with 1 mL chloroform:isopropanol 6:1. Evaporate the eluate to dryness under a stream of nitrogen at 30°, reconstitute the residue in 100  $\mu$ L mobile phase, inject a 15  $\mu$ L aliquot.

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**HPLC VARIABLES**

**Column:** 150  $\times$  4.6 5  $\mu$ m Supelcosil-LC-8

**Mobile phase:** MeCN:water 20:80

**Flow rate:** 3.3

**Injection volume:** 15

**Detector:** UV 208

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**CHROMATOGRAM**

**Retention time:** 4.08

**Internal standard:** tolylphenobarbital (7.57)

**Limit of detection:** 50-100 ng/mL

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**OTHER SUBSTANCES**

**Extracted:** theophylline, caffeine, barbital, ethosuximide, primidone, carbamazepinediol, phenacemide, methyprylon, nirvanol, phenobarbital, carbamazepine epoxide, mephentoin, pentobarbital, amobarbital, carbamazepine, glutethimide, phenytoin, secobarbital, methaqualone

**Noninterfering:** acetaminophen, amikacin, amitriptyline, clonazepam, cyclosporine, desipramine, diazepam, digoxin, disopyramide, gentamicin, imipramine, lidocaine, methotrexate, N-acetylprocainamide, netilmicin, nortriptyline, procainamide, quinidine, salicylic acid, sulfamethoxazole, tobramycin, trimethoprim, valproic acid, p-hydroxyphenobarbital, vancomycin

**Interfering:** butabarbital

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**KEY WORDS**

plasma; SPE

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**REFERENCE**

Svinarov,D.A.; Dotchev,D.C. Simultaneous liquid-chromatographic determination of some bronchodilators, anticonvulsants, chloramphenicol, and hypnotic agents, with Chromosorb P columns used for sample preparation, *Clin.Chem.*, **1989**, *35*, 1615-1618.

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**SAMPLE**

**Matrix:** blood

**Sample preparation:** 100  $\mu$ L Serum + 500  $\mu$ L 220  $\mu$ g/mL 4-nitroacetanilide in ethyl acetate, vortex for 30 s, centrifuge at 7000 g for 1 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 100  $\mu$ L mobile phase, inject a 10  $\mu$ L aliquot.

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**HPLC VARIABLES**

**Column:** 33  $\times$  4.6 3  $\mu$ m C18 (Perkin-Elmer)

**Mobile phase:** Isopropanol:100 mM pH 5.0 sodium acetate buffer 2:98

**Flow rate:** 2

**Injection volume:** 10

**Detector:** UV 278

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## CHROMATOGRAM

**Retention time:** 6.32

**Internal standard:** 4-nitroacetanilide (8.27)

**Limit of quantitation:** 10000 ng/mL

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## OTHER SUBSTANCES

**Extracted:** caffeine

**Simultaneous:** theobromine, theophylline, diphylline, chloramphenicol 3-monosuccinate

**Noninterfering:** acetaminophen, N-acetylprocainamide, amikacin, amitriptyline, carbamazepine, cyclosporine, digoxin, desipramine, disopyramide, ethosuximide, gentamicin, imipramine, lidocaine, lithium, methotrexate, netilmicin, nortriptyline, phenobarbital, phenytoin, primidone, procainamide, quinidine, salicylic acid, theophylline, tobramycin, valproic acid, vancomycin

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## KEY WORDS

serum

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## REFERENCE

Markin,R.S.; Wadman,M.C.; Bottjen,P.L.; Haven,M.C.; Huth,J.A. Short-column liquid chromatographic assay for caffeine and chloramphenicol in serum, *J.Chromatogr.*, **1990**, 525, 464–470.

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## SAMPLE

**Matrix:** blood

**Sample preparation:** 100  $\mu$ L Serum + 100  $\mu$ L 20  $\mu$ g/mL hydroxyethyltheophylline in 2 M perchloric acid, vortex, centrifuge 5 min, inject 50  $\mu$ L aliquot of supernatant.

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## HPLC VARIABLES

**Column:** 125  $\times$  4 LiChroSpher RP-8 5  $\mu$ m

**Mobile phase:** MeOH:buffer 15:85 (Buffer was 5 mL 2 M sodium acetate + 845 mL water, pH adjusted to 4.0 with acetic acid.)

**Column temperature:** 45

**Flow rate:** 1.5

**Injection volume:** 50

**Detector:** UV 282

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## CHROMATOGRAM

**Retention time:** 18

**Internal standard:** hydroxyethyltheophylline (5.6)

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## OTHER SUBSTANCES

**Simultaneous:** theophylline, caffeine

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## KEY WORDS

serum

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## REFERENCE

Hannak,D.; Haux,P.; Scharbert,F.; Kattermann,R. Liquid chromatographic analysis of phenobarbital, phenytoin, and theophylline, *Wien.Klin.Wochenschr.Suppl.*, **1992**, 191, 27–31.

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## SAMPLE

**Matrix:** blood, CSF

**Sample preparation:** 200  $\mu$ L Serum, plasma, or CSF + 300  $\mu$ L reagent. Flush column A to waste with 500  $\mu$ L 500 mM ammonium sulfate, inject sample onto column A, flush column A to waste with 500  $\mu$ L 500 mM ammonium sulfate, elute the contents of column

A onto column B with mobile phase, monitor the effluent from column B. (Reagent was 8.05 M guanidine hydrochloride and 1.02 M ammonium sulfate in water.)

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**HPLC VARIABLES**

**Column:** A 30 × 2.1 40 µm preparative grade C18 (Analytichem); B 250 × 4.6 10 µm Partisil C8

**Mobile phase:** Gradient. A was 50 mM pH 4.5 KH<sub>2</sub>PO<sub>4</sub>. B was MeCN:isopropanol 80:20. A: B 90:10 for 1 min, to 30:70 over 15 min, maintain at 30:70 for 4 min.

**Column temperature:** 50

**Flow rate:** 1.5

**Detector:** UV 280 for 5 min then UV 254

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**CHROMATOGRAM**

**Retention time:** 10.52

**Internal standard:** heptanophenone (19.2)

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**OTHER SUBSTANCES**

**Extracted:** acetazolamide, ampicillin, bromazepam, caffeine, carbamazepine, chlorothiazide, diazepam, droperidol, ethionamide, furosemide, isoniazid, methadone, penicillin G, phenobarbital, phenytoin, prazepam, propoxyphene, pyrazinamide, rifampin, trimeprazine, trimethoprim

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**KEY WORDS**

plasma; serum; column-switching

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**REFERENCE**

Seifart,H.I.; Kruger,P.B.; Parkin,D.P.; van Jaarsveld,P.P.; Donald,P.R. Therapeutic monitoring of anti-tuberculosis drugs by direct in-line extraction on a high-performance liquid chromatography system, *J.Chromatogr.*, **1993**, 619, 285-290.

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**SAMPLE**

**Matrix:** blood, CSF, gastric contents, urine

**Sample preparation:** 200 µL Serum, urine, CSF, or gastric fluid + 300 µL reagent. Flush column A to waste with 500 µL 500 mM ammonium sulfate, inject sample onto column A, flush column A to waste with 500 µL 500 mM ammonium sulfate, backflush the contents of column A onto column B with mobile phase, monitor the effluent from column B. (Reagent was 8.05 M guanidine HCl and 1.02 M ammonium sulfate in water.)

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**HPLC VARIABLES**

**Column:** A 40 µm preparative grade C18 (Analytichem); B 75 × 2.1 pellicular C18 (Whatman) + 250 × 4.6 5 µm C8 end-capped (Whatman)

**Mobile phase:** Gradient. A was 50 mM pH 4.5 KH<sub>2</sub>PO<sub>4</sub>. B was MeCN:isopropanol 80:20. A: B 90:10 for 1 min, to 30:70 over 20 min.

**Column temperature:** 50

**Flow rate:** 1.5

**Detector:** UV 220

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**CHROMATOGRAM**

**Retention time:** 9.92

**Internal standard:** heptanophenone (19)

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**OTHER SUBSTANCES**

**Extracted:** acetaminophen, allobarbitol, azinphos, barbital, brallobarbitone, bromazepam, butethal, caffeine, carbamazepine, carbaryl, cephaloridine, chlordiazepoxide, chlorothiazide, chlorvinphos, clothiapine, cocaine, coomassie blue, desipramine, diazepam, diphenhydramine, dipipanone, ethylbromphos, flufenamic acid, formothion, griseofulvin, indomethacin, lidocaine, lorazepam, malathion, medazepam, midazolam, oxazepam, paraoxon, penicillin G, pentobarbital, prazepam, propoxyphene, prothiophos, quinine, salicylic acid, secobarbital, strychnine, sulfamethoxazole, theophylline, thiopental, thioridazine, trimethoprim

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**KEY WORDS**

serum; column-switching

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**REFERENCE**

Kruger,P.B.; Albrecht,C.F.De V.; Jaarsveld,P.P. Use of guanidine hydrochloride and ammonium sulfate in comprehensive in-line sorption enrichment of xenobiotics in biological fluids by high-performance liquid chromatography, *J.Chromatogr.*, **1993**, 612, 191–198.

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**SAMPLE**

**Matrix:** blood, urine

**Sample preparation:** 200  $\mu$ L Plasma (dog) or urine (rat) + 50  $\mu$ L ammonium hydroxide: water 50:50 + 1 mL MTBE, extract, centrifuge. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 100  $\mu$ L mobile phase, inject a 20  $\mu$ L aliquot.

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**HPLC VARIABLES**

**Column:** 80  $\times$  4.6 3.65  $\mu$ m Zorbax Rx-SIL (similar to Zorbax SB-C8 (Mac-Mod Analytical))  
**Mobile phase:** MeCN:buffer 25:75 (Buffer was 0.1% trifluoroacetic acid adjusted to pH 3 with ammonium hydroxide.)

**Flow rate:** 1

**Injection volume:** 20

**Detector:** UV 278

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**CHROMATOGRAM**

**Retention time:** 4

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**KEY WORDS**

plasma; dog; rat

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**REFERENCE**

Kirkland,K.M.; McCombs,D.A.; Kirkland,J.J. Rapid, high-resolution high-performance liquid chromatographic analysis of antibiotics, *J.Chromatogr.A*, **1994**, 660, 327–337.

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**SAMPLE**

**Matrix:** blood, urine

**Sample preparation:** Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50  $\mu$ L MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood)  $\mu$ L aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200–350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

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**HPLC VARIABLES**

**Guard column:** 20 mm long Symmetry C18

**Column:** 250  $\times$  4.6 5  $\mu$ m Symmetry C8 (Waters)

**Mobile phase:** Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A: B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

**Column temperature:** 30

**Flow rate:** 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)

**Injection volume:** 10–30

**Detector:** UV 200.5

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**CHROMATOGRAM****Retention time:** 14.105

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**KEY WORDS**whole blood

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**REFERENCE**

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, 763, 149–163.

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**SAMPLE****Matrix:** feces**Sample preparation:** Homogenize (Polytron) with three volumes water, centrifuge at 4° at 23000 g for 20 min, inject an aliquot of the supernatant.

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**HPLC VARIABLES****Column:** 200 × 4.6 5 µm ODS2 (SFCC)**Mobile phase:** MeOH:100 mM pH 4.4 citrate buffer 20:80**Flow rate:** 0.8**Detector:** UV 280

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**CHROMATOGRAM****Retention time:** 57.9

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**OTHER SUBSTANCES****Extracted:** metabolites

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**KEY WORDS**duck; radiolabeled

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**REFERENCE**

Cravedi,J.P.; Baradat,M.; Debrauwer,L.; Alary,J.; Tulliez,J.; Bories,G. Evidence for new metabolic pathways of chloramphenicol in the duck, *Drug Metab.Dispos.*, **1994**, 22, 578–583.

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**SAMPLE****Matrix:** formulations

**Sample preparation:** Add capsule contents to water, extract with three 20 mL portions of ethyl acetate. Dry the extracts over potassium carbonate and evaporate them to dryness under reduced pressure. Add 120 mg of this product to 2.5 mL 5% HCl, heat at 95° for 2.5 h, cool to room temperature, adjust pH to 11 with concentrated NaOH, extract five times with 7 mL portions of ethyl acetate. Combine the extracts and dry them over potassium carbonate, evaporate to dryness, prepare a 10 mg/mL solution in MeOH. Add a 50 µL aliquot to 150 µL 10 mg/mL 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl isothiocyanate (TAGIT) in MeCN, let stand at room temperature for 20 min, add 2 µL 2-aminoethanol, let stand for 10 min, add 50 µL 300 mM pH 3 ammonium phosphate, inject a 2-5 µL aliquot. (Chloramphenicol is de-acylated to 2-amino-1-(p-nitrophenyl)-1,3-propanediol which is then derivatized. The diastereomer obtained from chloramphenicol (1R,2R) can be resolved from the other possible diastereomers.)

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**HPLC VARIABLES****Column:** 150 × 3.9 Nova-pak ODS**Mobile phase:** MeOH:water 42:58**Flow rate:** 1**Injection volume:** 2-5**Detector:** UV 254

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**CHROMATOGRAM**

**Retention time:** 12 (1R,2R), 18 (1S,2S), 22 (1R,2S or 1S,2R), 25 (1R,2S or 1S,2R)

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**KEY WORDS**

derivatization; capsules

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**REFERENCE**

Gal,J.; Meyer-Lehnert,S. Reversed-phase liquid chromatographic separation of enantiomeric and diastereomeric bases related to chloramphenicol and thiamphenicol, *J.Pharm.Sci.*, **1988**, 77, 1062–1065.

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**SAMPLE**

**Matrix:** milk, tissue

**Sample preparation:** Prepare a SPE extraction column by placing 1.2 g silica gel (40  $\mu\text{m}$ , Baker) in a 3 mL filtration column (Baker), condition with 8 mL ethyl acetate:hexane 40:60, do not allow to dry. Tissue. 10 g Ground tissue + 20 mL ethyl acetate, stir thoroughly with a glass rod, sonicate below 40° for 15 min, allow to settle, decant through 5 g anhydrous sodium sulfate on filter paper (S & S 589.1), repeat extraction, wash solids with 10 mL ethyl acetate. Combine all organic layers, add 60 mL hexane, stir thoroughly, allow to stand for 5 min, filter (S & S 589.3 paper), wash filter with 10 mL hexane. Combine all organic layers and pass through the SPE column at 8/10 mL/min, wash with 10 mL hexane, dry in a stream of nitrogen for 20 min, elute with four 1 mL portions of MeOH. Evaporate the eluate to dryness under a stream of nitrogen, reconstitute the residue in 1 mL mobile phase, vortex for 30 s, inject a 50  $\mu\text{L}$  aliquot. Milk. 10 g Homogenized milk + 0.4 g citric acid monohydrate + 30 mL ethyl acetate, shake mechanically for 15 min, centrifuge at 2300 g for 10 min, filter (paper) the organic layer, repeat extraction with 10 mL ethyl acetate, filter (paper), wash filter with 10 mL ethyl acetate, add 60 mL hexane to filtrate, stir thoroughly, allow to stand for 5 min, filter (S & S 589.3 paper), wash filter with 10 mL hexane. Combine all organic layers and pass through the SPE column at 8/10 mL/min, wash with 10 mL hexane, dry in a stream of nitrogen for 20 min, elute with four 1 mL portions of MeOH. Evaporate the eluate to dryness under a stream of nitrogen, reconstitute the residue in 1 mL mobile phase, vortex for 30 s, inject a 50  $\mu\text{L}$  aliquot. (*J.Chrom.* 1991, 566, 173)

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**HPLC VARIABLES**

**Guard column:** 10  $\times$  2.1 reversed-phase (Chrompack)

**Column:** Two 100  $\times$  3 5  $\mu\text{m}$  ChromSpher C8 glass columns in series (Chrompack)

**Mobile phase:** MeCN:10 mM pH 4.3 sodium acetate buffer 25:75

**Flow rate:** 0.8

**Injection volume:** 50

**Detector:** UV 280

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**CHROMATOGRAM**

**Retention time:** 5

**Limit of quantitation:** 1 ng/g (milk), 10 ng/g (tissue)

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**KEY WORDS**

pig; cow; muscle; SPE

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**REFERENCE**

Haagsma,N.; Schreuder,C.; Rensen,E.R.A. Rapid sample preparation method for the determination of chloramphenicol in swine muscle by high-performance liquid chromatography, *J.Chromatogr.*, **1986**, 363, 353–359.

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**SAMPLE**

**Matrix:** solutions

**Sample preparation:** Centrifuge and filter cell solutions (0.22  $\mu\text{m}$ ), inject an aliquot.



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**HPLC VARIABLES**

**Guard column:** Guard-PAK C18 (Waters)

**Column:** 150 × 3.9 5 µm NOVA PAK C18

**Mobile phase:** MeOH:50 mM pH 6.0 KH<sub>2</sub>PO<sub>4</sub> 50:50

**Flow rate:** 0.6

**Detector:** UV 313

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**CHROMATOGRAM**

**Retention time:** 4.2

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**REFERENCE**

Koga, H. High-performance liquid chromatography measurement of antimicrobial concentrations in polymorphonuclear leukocytes, *Antimicrob. Agents Chemother.*, **1987**, *31*, 1904–1908.

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**SAMPLE**

**Matrix:** solutions

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**HPLC VARIABLES**

**Column:** 250 × 4.6 Zorbax RX

**Mobile phase:** Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.

**Column temperature:** 30

**Flow rate:** 2

**Detector:** UV 210

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**OTHER SUBSTANCES**

**Also analyzed:** acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepam, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarboxtyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephénytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyl dopa, methyl dopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbu-

tazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxazole, sulfanilamide, sulfapyridine, sulfasoxazole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranlycypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripeleminamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

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## REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

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## SAMPLE

**Matrix:** tissue

**Sample preparation:** Condition a 3 mL silica SPE cartridge with 10 mL ethyl acetate:hexane (4:10 ?). Vortex 3 g fatty liver and 3 mL water for 1 min, add 15 mL hexane, stir at 40 rpm for 10 min. Centrifuge at 2400 g for 5 min and discard the supernatant. Add 12 mL ethyl acetate, vortex twice for 1 min periods at a 15 min interval, centrifuge at 4000 g for 5 min, decant 8 mL organic phase through glass wool covered by 1 g anhydrous sodium sulfate, wash twice with 2 mL portions of ethyl acetate. Add 30 mL hexane to the combined extracts. Add to the SPE cartridge, rinse the flask and sodium sulfate with 10 mL ethyl acetate:hexane 4:10, add the rinse to the SPE cartridge, wash with 10 mL hexane, dry under vacuum for 2 min, elute with 5 mL 50 mM pH 10 potassium phosphate buffer. Add 20 mL ethyl acetate to the eluate, vortex, centrifuge at 2400 g for 10 min. Evaporate 18 mL of the ethyl acetate phase to dryness under reduced pressure, reconstitute the oily residue in 1.2 mL hexane:chloroform 50:50 (Caution! Chloroform is a carcinogen!). Add 800  $\mu$ L water, stir at 35 rpm for 5 min, centrifuge at 3300 g for 10 min, inject an aliquot.

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## HPLC VARIABLES

**Guard column:** 4  $\times$  4  $\mu$ m Nova-Pak C18

**Column:** 150  $\times$  3.9  $\mu$ m Nova-Pak C18

**Mobile phase:** MeCN:5 mM pH 7.9 diammonium hydrogen phosphate buffer 19:81

**Flow rate:** 1

**Detector:** UV 278

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## CHROMATOGRAM

**Retention time:** 8.7

**Limit of detection:** 400 pg/g

**Limit of quantitation:** 800 pg/g

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## KEY WORDS

SPE; liver

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## REFERENCE

Roudaut,B. High-performance liquid chromatography with UV detection and scanning UV confirmation of chloramphenicol in fatty liver, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 1097–1105.

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**SAMPLE****Matrix:** tissue**Sample preparation:** Grind muscle, liver or kidney with a kitchen mixer for 1 min. Weigh out 5 g ground tissue, add 2 mL water, vortex for 1 min, let stand for 10 min, add 6 mL ethyl acetate, vortex for 1 min, centrifuge at 4000 rpm for 5 min. Remove 4.2 mL of the upper organic layer and evaporate it to dryness under reduced pressure at 30°, suspend the residue in 1.4 mL hexane:chloroform 50:50, add 0.7 mL water, stir (Heidolf stirrer) at 35 rpm for 5 min, centrifuge at 4000 rpm for 10 min, repeat if necessary, inject a 200  $\mu$ L aliquot of the supernatant

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**HPLC VARIABLES****Guard column:** 4  $\times$  4 C8**Column:** 150  $\times$  4.6 Novapack C18**Mobile phase:** MeCN:0.66 g/L pH 7.9 (NH<sub>4</sub>)<sub>2</sub>HPO<sub>4</sub> 29:71 (muscle) or 18:82 (liver) or MeCN:0.66 g/L (NH<sub>4</sub>)<sub>2</sub>HPO<sub>4</sub> adjusted to pH 8.3 with 25% ammonia solution 29:71 (kidney) (Flush daily with MeCN:0.1% sulfuric acid 18:82.)**Flow rate:** 1**Injection volume:** 200**Detector:** UV 278

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**CHROMATOGRAM****Limit of detection:** 1 ng/g

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**KEY WORDS**

cow; muscle; liver; kidney

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**REFERENCE**Sanders,P.; Guillot,P.; Dagorn,M.; Delmas,J.M. Liquid chromatographic determination of chloramphenicol in calf tissues: studies of stability in muscle, kidney, and liver, *J.Assoc. Off. Anal. Chem.*, **1991**, 74, 483-486.

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**SAMPLE****Matrix:** tissue**Sample preparation:** Condition a Sep-Pak silica cartridge with 5 mL MeCN:water 20:80, 5 mL MeCN, and 5 mL dichloromethane, then dry with a stream of nitrogen for 30 min. Homogenize tissue (Sorvall Omnimixer). 10 g Homogenized tissue + 30 g anhydrous sodium sulfate + 30 mL MeCN, homogenize for 1 min, centrifuge at 4000 rpm for 10 min, remove the MeCN layer, extract the aqueous layer twice more with 30 mL MeCN. Combine the MeCN layers and wash them twice with 60 mL portions of n-hexane with vigorous shaking for 30 s each time. Evaporate the MeCN layer to dryness under a stream of nitrogen at 50°, dissolve the residue in dichloromethane, add the sample extract to the SPE cartridge, wash with two 5 mL aliquots of dichloromethane, dry cartridge with a stream of nitrogen for 30 min, elute with 5 mL MeCN:water 20:80. Add the eluate to 1 mL ethyl acetate, shake, remove the upper organic layer, repeat the extraction twice more, combine the organic layers and evaporate them to dryness under a stream of nitrogen at 50°, reconstitute the residue in 1 mL mobile phase, inject a 50  $\mu$ L aliquot.

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**HPLC VARIABLES****Column:** 4  $\mu$ m Nova-Pak C18 radial compression module**Mobile phase:** MeCN:buffer 30:70 (Prepare buffer by diluting 1 M pH 4.8 sodium acetate buffer 1:100 with water.)**Flow rate:** 1**Injection volume:** 50**Detector:** UV 278

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**CHROMATOGRAM****Retention time:** 7.5

**Limit of detection:** 10 ppb

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**KEY WORDS**

chicken; muscle; SPE

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**REFERENCE**

Ramos,M.; Reuvers,T.; Aranda,A.; Gómez,J. Determination of chloramphenicol in chicken muscle by high performance liquid chromatography and UV-diode array detection, *J.Liq.Chromatogr.*, **1994**, 17, 385–401.

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**SAMPLE**

**Matrix:** tissue

**Sample preparation:** Homogenize tissue with an Omni-mixer (Sorval). Sonicate 10 g muscle or 5 g liver with 40 mL water for 8 min, centrifuge for 10 min, add 20 mL of the supernatant to a Chem Elut CE 1020 SPE cartridge (Analytichem), allow to equilibrate for 15 min, elute with 50 mL ethyl acetate. Evaporate the eluate to dryness under reduced pressure, reconstitute with 500  $\mu$ L water, add 1 mL toluene, vortex gently, centrifuge, discard the organic layer, repeat wash. Filter (0.45  $\mu$ m) the aqueous phase, inject a 100  $\mu$ L aliquot of the filtrate.

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**HPLC VARIABLES**

**Column:** 150  $\times$  4.6  $\mu$ m Hypersil RP-18

**Mobile phase:** MeCN:50 mM pH 3 triethylamine phosphate buffer 21:79

**Flow rate:** 1

**Injection volume:** 100

**Detector:** UV 278

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**CHROMATOGRAM**

**Retention time:** 11

**Limit of detection:** 2 ng/g

**Limit of quantitation:** 8 ng/g

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**KEY WORDS**

liver; muscle; quail; pheasant; mallard; SPE

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**REFERENCE**

Di Pietra,A.M.; Piazza,V.; Andrisano,V.; Cavrini,V. HPLC determination of chloramphenicol and thiamphenicol residues in gamebird meats, *J.Liq.Chromatogr.*, **1995**, 18, 3529–3543.

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**SAMPLE**

**Matrix:** tissue

**Sample preparation:** Dry 10 g homogenized (Ultra-Turrax) tissue and 5 g sand at 60° for 5 h, mix with 20 mL MeCN, homogenize (Ultra-Turrax) for 3 min, centrifuge at 2980 g for 5 min, decant the supernatant, repeat the extraction. Combine the extracts and evaporate them to dryness, dissolve the residue in 1 mL MeCN, add to a Sep-Pak C18 cartridge, rinse tube twice with 1 mL portions of MeCN, add rinses to the SPE cartridge, elute with 4 mL MeCN. Evaporate the eluate to dryness and reconstitute the residue in ethyl acetate, pass through a silica SPE cartridge, elute with ethyl acetate. Evaporate the eluate to dryness under reduced pressure, reconstitute in 500  $\mu$ L n-hexane and 500  $\mu$ L mobile phase, inject a 50  $\mu$ L aliquot of the lower aqueous phase onto column A and elute to waste with mobile phase at 0.7 mL/min, divert the fraction containing chloramphenicol onto column B while eluting column B to waste. When all the chloramphenicol has eluted from column A remove column A from the circuit, backflush the contents of column B onto column C with mobile phase at 0.9 mL/min, monitor the effluent from column C.

**HPLC VARIABLES**

**Column:** A  $150 \times 4.6$  5  $\mu\text{m}$  LC-HISEP (Supelco); B  $150 \times 4.6$  5  $\mu\text{m}$  Supelcosil LC-18; C  $250 \times 4.6$  5  $\mu\text{m}$  Supelcosil LC-18

**Mobile phase:** MeCN:THF:water 80:2:18

**Flow rate:** 0.7, 0.9

**Injection volume:** 50

**Detector:** UV 278

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**CHROMATOGRAM**

**Retention time:** 26

**Limit of quantitation:** 2 ng/g

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**KEY WORDS**

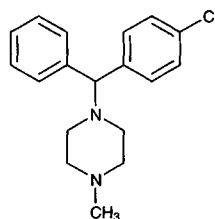
column-switching; pig; liver; kidney; muscle; fat; skin; turkey; trout; fish; SPE

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**REFERENCE**

Hummert,C.; Luckas,B.; Siebenlist,H. Determination of chloramphenicol in animal tissue using high-performance liquid chromatography with a column-switching system and ultraviolet detection, *J.Chromatogr.B*, **1995**, 668, 53–58.

# Chlorcyclizine



**Molecular formula:**  $C_{18}H_{21}ClN_2$

**Molecular weight:** 300.83

**CAS Registry No.:** 82-93-9, 1620-21-9 (HCl)

**Merck Index:** 2128

**Lednicher No.:** 1 58

## SAMPLE

**Matrix:** blood, CSF

**Sample preparation:** Plasma. Centrifuge blood at 7000 rpm, decant 100  $\mu$ L plasma. Mix 100  $\mu$ L plasma with 200  $\mu$ L acetone, centrifuge at 7000 rpm for 5 min. Evaporate the supernatant under a stream of nitrogen, reconstitute the residue with mobile phase, inject an aliquot. CSF. Add 25  $\mu$ L water to 25  $\mu$ L CSF, mix with 50  $\mu$ L acetone, centrifuge at 7000 rpm for 5 min, decant the supernatant, evaporate under a stream of nitrogen, reconstitute the residue with mobile phase, inject an aliquot.

## HPLC VARIABLES

**Column:**  $\mu$ Bondapak C18

**Mobile phase:** MeCN:water 32:68 containing 0.05% trifluoroacetic acid

## KEY WORDS

plasma; rat; pharmacokinetics

## REFERENCE

Chou, K.-J.; Donovan, M.D. Distribution of antihistamines into the CSF following intranasal delivery, *Biopharm. Drug Dispos.*, **1997**, *18*, 335-346.

## SAMPLE

**Matrix:** solutions

**Sample preparation:** Prepare a 10  $\mu$ g/mL solution in MeOH, inject a 20  $\mu$ L aliquot.

## HPLC VARIABLES

**Column:** 125  $\times$  4.9 Spherisorb S5W silica

**Mobile phase:** MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7

**Flow rate:** 2

**Injection volume:** 20

**Detector:** E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

## CHROMATOGRAM

**Retention time:** 3.1

## OTHER SUBSTANCES

**Also analyzed:** acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzocetamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyrindamole, disopyramide,

dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamlamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdiazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypropazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozone, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxy-metacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thiopropazine, thioridazine, thiothixene, thonzylamine, timolol, tocanide, tolpropamine, tolycaine, tranlycypromine, trazodone, trifluoperazine, trifluoperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripeleminamine, triprolidine, tryptamine, verapamil, xylometazoline

## REFERENCE

Jane, I.; McKinnon, A.; Flanagan, R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, 323, 191-225.

## SAMPLE

**Matrix:** solutions

**Sample preparation:** Dissolve in MeOH:water 1:1 at a concentration of 50 µg/mL, inject a 10 µL aliquot.

## HPLC VARIABLES

**Column:** 300 × 3.9 10 µm µBondapak C18

**Mobile phase:** MeOH:acetic acid:triethylamine:water 60:1.5:0.5:38

**Flow rate:** 1.5

**Injection volume:** 10

**Detector:** UV

## CHROMATOGRAM

**Retention time:** k' 3.59

## REFERENCE

Roos, R.W.; Lau-Cam, C.A. General reversed-phase high-performance liquid chromatographic method for the separation of drugs using triethylamine as a competing base, *J.Chromatogr.*, **1986**, 370, 403-418.

## SAMPLE

**Matrix:** solutions

## HPLC VARIABLES

**Column:** 250 × 4.6 cellulose tris(4-tert-butylphenylcarbamate)

**Mobile phase:** Hexane:isopropanol 98:2

**Flow rate:** 0.5

**Detector:** UV

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## CHROMATOGRAM

**Retention time:** 9.5 (+), 10.5 (-)

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## KEY WORDS

chiral

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## REFERENCE

Okamoto, Y.; Aburatani, R.; Hatano, K.; Hatada, K. Optical resolution of racemic drugs by chiral HPLC on cellulose and amylose tris(phenylcarbamate) derivatives, *J. Liq. Chromatogr.*, **1988**, *11*, 2147–2163.

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## SAMPLE

**Matrix:** solutions

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## HPLC VARIABLES

**Guard column:** Supelguard (Supelco)

**Column:** 150 × 4.6 5 µm Supelcosil LC-8-DB

**Mobile phase:** MeCN:MeOH:buffer 19:28:53 (Buffer was 50 mM KH<sub>2</sub>PO<sub>4</sub> containing 0.2% triethylamine, pH 2.5.)

**Flow rate:** 1.5

**Injection volume:** 10

**Detector:** UV 254

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## CHROMATOGRAM

**Retention time:** 9

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## OTHER SUBSTANCES

**Simultaneous:** chlorpheniramine, clonidine, diphenhydramine, promethazine, pyrilamine, triprolidine

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## REFERENCE

*Supelco Catalog*, **1994**, 768.

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## SAMPLE

**Matrix:** solutions

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## HPLC VARIABLES

**Column:** 250 × 4.6 5 µm Vydac 201HS54 C18

**Mobile phase:** Gradient MeCN:25 mM pH 3.6 phosphate buffer from 20:80 to 70:30 over 20 min

**Flow rate:** 1.5

**Detector:** UV 220 (from Vydac Applications Brochure)

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## CHROMATOGRAM

**Retention time:** 11

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## OTHER SUBSTANCES

**Simultaneous:** tripeleennamine, triprolidine, cyclizine, methaphenilene, pyrrobutamine, meclizine, buclizine

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## REFERENCE

*Vydac HPLC Catalog*, **1994-5**,



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**SAMPLE**

**Matrix:** solutions

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**HPLC VARIABLES**

**Column:** 250 × 4.6 5 µm Supelcosil LC-DP (A) or 250 × 4.5 µm LiChrospher 100 RP-8 (B)

**Mobile phase:** MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)

**Flow rate:** 0.6

**Injection volume:** 25

**Detector:** UV 229

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**CHROMATOGRAM**

**Retention time:** 14.70 (A), 6.89 (B)

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**OTHER SUBSTANCES**

**Also analyzed:** acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlor-diazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephénytoin, mepivacaine, mesoridazine, metaproterenol, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyl-dopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfipyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocinide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

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**KEY WORDS**

also details of plasma extraction

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**REFERENCE**

Koves, E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, 692, 103-119.

# Chlordiazepoxide

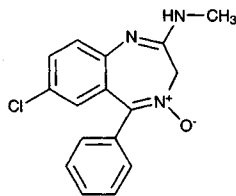
**Molecular formula:** C<sub>16</sub>H<sub>14</sub>ClN<sub>3</sub>O

**Molecular weight:** 299.76

**CAS Registry No.:** 58-25-3, 438-41-5 (HCl)

**Merck Index:** 2132

**Lednicer No.:** 1 365



## SAMPLE

**Matrix:** blood

**Sample preparation:** 500  $\mu$ L Serum + 20  $\mu$ L 20  $\mu$ g/mL IS + 200  $\mu$ L 1 M potassium carbonate + 3 mL chloroform, mix for 2 min, centrifuge at 1200 g for 5 min, aspirate aqueous phase. Evaporate the organic phase under a stream of nitrogen at 40°. Dissolve the residue in 100  $\mu$ L mobile phase, inject a 20  $\mu$ L aliquot. (Caution! Chloroform is a carcinogen!)

## HPLC VARIABLES

**Column:** 100  $\times$  4.6 2  $\mu$ m TSK gel Super-ODS (A) or 100  $\times$  4.6 5  $\mu$ m Hypersil ODS-C18 (B)

**Mobile phase:** MeCN:5 mM pH 6 NaH<sub>2</sub>PO<sub>4</sub> 45:55

**Flow rate:** 0.65

**Injection volume:** 20

**Detector:** UV 254

## CHROMATOGRAM

**Retention time:** 21.0 (A), 73.1 (B)

**Internal standard:** diazepam (29.8 (A), 77.5 (B))

**Limit of quantitation:** 1 ng/mL (A)

## OTHER SUBSTANCES

**Extracted:** bromazepam, clonazepam, estazolam, etizolam, flutazolam, haloxazolam, lorazepam, nitrazepam, oxazolam, triazolam

**Simultaneous:** alprazolam

**Noninterfering:** barbitol, carbamazepine, cloxazolam, ethosuximide, hexobarbital, mexazolam, oxazepam, pentobarbital, phenobarbital, phenytoin, primidone, trimethadione

## KEY WORDS

serum

## REFERENCE

Tanaka,E.; Terada,M.; Misawa,.; Wakasugi,C. Simultaneous determination of twelve benzodiazepines in human serum using a new reversed-phase chromatographic column on a 2- $\mu$ m porous microspherical silica gel, *J.Chromatogr.B*, **1996**, 682, 173-178.

## SAMPLE

**Matrix:** blood

**Sample preparation:** 200  $\mu$ L Serum + 200  $\mu$ L 50  $\mu$ g/mL hexobarbital in MeCN + 25  $\mu$ L glacial acetic acid, vortex for 10 s, centrifuge for 1 min, inject a 30-100  $\mu$ L aliquot of the supernatant.

## HPLC VARIABLES

**Column:**  $\mu$ Bondapak C18

**Mobile phase:** Gradient. MeCN:7.5 g/L NaH<sub>2</sub>PO<sub>4</sub> adjusted to pH 3.2 with phosphoric acid 5:95 to 22:78 over 24 min, to 45:55 over 10 min, maintain at 45:55 for 5 min. Re-equilibrate with 5:95 for 5 min.

**Column temperature:** 50

**Flow rate:** 3

**Injection volume:** 30-100

**Detector:** UV 210

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## CHROMATOGRAM

**Retention time:** 19.6

**Internal standard:** hexobarbital (20.6)

**Limit of detection:** 200-2000 ng/mL

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## OTHER SUBSTANCES

**Extracted:** acetaminophen, amobarbital, butabarbital, butalbital, diazepam, ethchlorvynol, flurazepam, glutethimide, methaqualone, methypylon, nitrazepam, pentobarbital, phenobarbital, phenytoin, primidone, salicylic acid, secobarbital, theophylline

**Simultaneous:** amitriptyline, caffeine, clomipramine, codeine, desipramine, ethotoin, imipramine, lidocaine, mesantoin, methsuximide, nirvanol, nortriptyline, oxazepam, procainamide, phenylpropanolamine, propranolol, quinidine

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## KEY WORDS

serum

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## REFERENCE

Kabra,P.M.; Stafford,B.E.; Marton,L.J. Rapid method for screening toxic drugs in serum with liquid chromatography, *J.Anal.Toxicol.*, **1981**, 5, 177-182.

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## SAMPLE

**Matrix:** blood

**Sample preparation:** 2 mL Plasma + 100  $\mu$ L 1  $\mu$ g/mL loxapine in isopropanol:diethylamine 99.9:0.1 + 250  $\mu$ L 25% potassium carbonate containing 0.1% diethylamine + 5 mL hexane:isoamyl alcohol 97:3, vortex for 30 s, centrifuge at 500 g for 3 min. Remove the organic layer and add it to 100  $\mu$ L 250 mM HCl, vortex for 30 s, inject a 50  $\mu$ L aliquot of the aqueous phase.

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## HPLC VARIABLES

**Guard column:** 50  $\times$  4.6 40  $\mu$ m C8 (Supelco)

**Column:** 250  $\times$  4.6 5  $\mu$ m Supelcosil C8

**Mobile phase:** MeCN:water:diethylamine:85% phosphoric acid 53.3:45.1:1:0.4, pH adjusted to 7.2 with NaOH or phosphoric acid

**Flow rate:** 2

**Injection volume:** 50

**Detector:** UV 254

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## CHROMATOGRAM

**Retention time:** k' 2.53

**Internal standard:** loxapine (k' 7.18)

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## OTHER SUBSTANCES

**Extracted:** amitriptyline, chlorpromazine, desipramine, desmethldiazepam, desmethyl-chlordiazepoxide, diazepam, doxepin, haloperidol, imipramine, nortriptyline, thiothixene

**Noninterfering:** molindone, perphenazine, trifluoperazine

**Interfering:** desmethyldoxepin, fluphenazine, oxazepam

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## KEY WORDS

plasma

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## REFERENCE

Kiel,J.S.; Abramson,R.K.; Morgan,S.L.; Voris,J.C. A rapid high performance liquid chromatographic method for the simultaneous measurement of six tricyclic antidepressants, *J.Liq.Chromatogr.*, **1983**, 6, 2761-2773.

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**SAMPLE**

**Matrix:** blood

**Sample preparation:** Inject 100-200  $\mu\text{L}$  plasma onto column A with mobile phase A and elute to waste, after 5 min backflush the contents of column A onto column B with mobile phase B, after 5 min remove column A from the circuit, elute column B with mobile phase B, monitor the effluent from column B. Wash column A with MeCN:water 60:40 at 1 mL/min for 6 min then re-equilibrate with pH 7.5 buffer for 10 min.

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**HPLC VARIABLES**

**Column:** A  $45 \times 4.12 \mu\text{m}$  TSK-gel G 3 PW (Tosohass); B  $75 \times 4.6$  Ultrasphere ODS C18 3  $\mu\text{m}$

**Mobile phase:** A 50 mM pH 7.5 phosphate buffer; B Gradient. A was MeCN. B was 65 mM  $\text{KH}_2\text{PO}_4$  + 1% diethylamine adjusted to pH 5.4 with phosphoric acid. A:B 22:78 for 5 min, to 25:75 over 10 min, to 60:40 over 15 min.

**Flow rate:** 1

**Injection volume:** 100-200

**Detector:** UV 230

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**CHROMATOGRAM**

**Retention time:** 22.8

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**OTHER SUBSTANCES**

**Extracted:** alprazolam, bromazepam, clobazam, clonazepam, clorazepate, clotiazepam, desmethyloclobazam, desmethyldiazepam, diazepam, estazolam, flunitrazepam, loflazepam, lorazepam, medazepam, nitrazepam, oxazepam, prazepam, temazepam, tetrazepam, tofisopam, triazolam

**Noninterfering:** carbamazepine, phenytoin, ethosuximide, phenobarbital, primidone, valproic acid

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**KEY WORDS**

plasma; column-switching

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**REFERENCE**

Lacroix, C.; Wojciechowski, F.; Danger, P. Monitoring of benzodiazepines (clobazam, diazepam and their main active metabolites) in human plasma by column-switching high-performance liquid chromatography, *J. Chromatogr.*, **1993**, 617, 285-290.

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**SAMPLE**

**Matrix:** blood

**Sample preparation:** Automated SPE by ASPEC system. Condition a C18 Clean-Up SPE cartridge (CEC 18111, Worldwide Monitoring) with 2 mL MeOH then 2 mL water. 1 mL Plasma + 1 mL 400 ng/mL protriptyline in water, vortex, add to column, wash with 3 mL water, wash with 3 mL 750 mL/L methanol. Elute with three aliquots of 300  $\mu\text{L}$  0.1 M ammonium acetate in MeOH. Add 0.5 mL 0.5 M NaOH and 4 mL 50 mL/L isopropanol in heptane to eluate, mix thoroughly. Allow 5 min for phase separation. Remove upper heptane phase and add it to 300  $\mu\text{L}$  0.1 M phosphoric acid (pH 2.5), mix, separate, inject a 100  $\mu\text{L}$  aliquot of the aqueous phase.

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**HPLC VARIABLES**

**Guard column:** LC-8-DB (Supelco)

**Column:**  $150 \times 4.6$  LC-8-DB (Supelco)

**Mobile phase:** MeCN:buffer 35:65 (Buffer was 10 mL/L triethylamine in water adjusted to pH 5.5 with glacial acetic acid.)

**Flow rate:** 2

**Injection volume:** 100

**Detector:** UV 228

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**CHROMATOGRAM****Retention time:** 3.6**Internal standard:** protriptyline (4)

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**OTHER SUBSTANCES**

**Extracted:** acetazolamide, amitriptyline, chlorimipramine, chlorpromazine, dextromethorphan, diazepam, diphenhydramine, doxepin, encainide, fentanyl, flecainide, fluoxetine, flurazepam, haloperidol, hydroxyethylflurazepam, imipramine, lidocaine, maprotiline, methadone, mexiletine, midazolam, norchlorimipramine, nordoxepin, nordiazepam, norfluoxetine, nortriptyline, pentazocine, propoxyphene, propranolol, protriptyline, quinidine, temazepam, trazodone, trimipramine, verapamil

**Noninterfering:** acetaminophen, acetylmorphine, amiodarone, amobarbital, amphetamine, bendroflumethiazide, benzocaine, benzoylecgonine, benzthiazide, butalbital, carbamazepine, chlorothiazide, clonazepam, cocaine, codeine, cotinine, cyclosporine, cyclothiazide, desalkylflurazepam, diamorphine, dicumerol, ephedrine, ethacrynic acid, ethanol, ethchlorvynol, ethosuximide, furosemide, glutethimide, hydrochlorothiazide, hydrocodone, hydroflumethiazide, hydromorphone, lorazepam, mephentermine, meprobamate, methamphetamine, metharbital, methoxsalen, methoxyphenteramine, methsuximide, methylcyclothiazide, metoprolol, MHPG, monoacetylmorphine, morphine, normethsuximide, oxazepam, oxycodone, oxymorphone, pentobarbital, phencyclidine, phenteramine, phenylephrine, phenytoin, polythiazide, primidone, prochlorperazine, salicylic acid, sulfanilamide, THC-COOH, theophylline, thiazolam, thiopental, thioridazine, tocinide, trichloromethiazide, trifluoperazine, valproic acid, warfarin

**Interfering:** desipramine, ibuprofen, methaqualone, norverapamil, promazine, propafenone

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**KEY WORDS**

plasma; SPE

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**REFERENCE**

Nichols, J.H.; Charlson, J.R.; Lawson, G.M. Automated HPLC assay of fluoxetine and norfluoxetine in serum, *Clin. Chem.*, **1994**, *40*, 1312-1316.

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**SAMPLE****Matrix:** blood

**Sample preparation:** 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform: isopropanol:n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 µL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 µL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

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**HPLC VARIABLES****Column:** 300 × 3.9 µm NovaPack C18

**Mobile phase:** MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH<sub>2</sub>PO<sub>4</sub>, adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)

**Column temperature:** 30**Flow rate:** 0.8**Injection volume:** 50**Detector:** UV 262

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**CHROMATOGRAM****Retention time:** 5.10**Limit of detection:** <120 ng/mL

## KEY WORDS

whole blood; plasma; interferences may occur—compounds(all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; videsine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phenacyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; aceprometazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluoperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thiopropazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tiocloamarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

## REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, 1995, 40, 254–262.

## SAMPLE

**Matrix:** blood, CSF, gastric contents, urine

**Sample preparation:** 200  $\mu$ L Serum, urine, CSF, or gastric fluid + 300  $\mu$ L reagent. Flush column A to waste with 500  $\mu$ L 500 mM ammonium sulfate, inject sample onto column A, flush column A to waste with 500  $\mu$ L 500 mM ammonium sulfate, backflush the contents of column A onto column B with mobile phase, monitor the effluent from column B. (Reagent was 8.05 M guanidine HCl and 1.02 M ammonium sulfate in water.)

## HPLC VARIABLES

**Column:** A 40  $\mu$ m preparative grade C18 (Analytichem); B 75  $\times$  2.1 pellicular C18 (Whatman) + 250  $\times$  4.6 5  $\mu$ m C8 end-capped (Whatman)

**Mobile phase:** Gradient. A was 50 mM pH 4.5  $\text{KH}_2\text{PO}_4$ . B was MeCN:isopropanol 80:20. A: B 90:10 for 1 min, to 30:70 over 20 min.

**Column temperature:** 50

**Flow rate:** 1.5

**Detector:** UV 220

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## CHROMATOGRAM

**Retention time:** 12.96

**Internal standard:** heptanophenone (19)

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## OTHER SUBSTANCES

**Extracted:** acetaminophen, allobarbitol, azinphos, barbitol, brallobarbitone, bromazepam, butethal, caffeine, carbamazepine, carbaryl, cephaloridine, chloramphenicol, chlorothiazide, chlorvinphos, clothiapine, cocaine, coomassie blue, desipramine, diazepam, diphenhydramine, dipipanone, ethylbromphos, flufenamic acid, formothion, griseofulvin, indomethacin, lidocaine, lorazepam, malathion, medazepam, midazolam, oxazepam, paraoxon, penicillin G, pentobarbital, prazepam, propoxyphene, prothiophos, quinine, salicylic acid, secobarbital, strychnine, sulfamethoxazole, theophylline, thiopental, thioridazine, trimethoprim

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## KEY WORDS

serum; column-switching

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## REFERENCE

Kruger,P.B.; Albrecht,C.F.De V.; Jaarsveld,P.P. Use of guanidine hydrochloride and ammonium sulfate in comprehensive in-line sorption enrichment of xenobiotics in biological fluids by high-performance liquid chromatography, *J.Chromatogr.*, **1993**, 612, 191-198.

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## SAMPLE

**Matrix:** blood, gastric contents, tissue, urine

**Sample preparation:** 1 mL Blood, urine, or gastric contents or 1 g tissue homogenate + 500  $\mu$ L buffer + 8 mL n-hexane:ethyl acetate 70:30, mix on a rotary mixer for 10 min, centrifuge at 3000 g for 8 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 100  $\mu$ L 12.5 mM NaOH in MeOH: water 50:50, inject a 50  $\mu$ L aliquot. (Buffer was 13.8 g potassium carbonate in 100 mL water, pH adjusted to 9.5 with concentrated HCl.)

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## HPLC VARIABLES

**Guard column:** 4  $\times$  4 30  $\mu$ m LiChrocart Aluspher RP-select B (Merck)

**Column:** 125  $\times$  4 5  $\mu$ m Aluspher RP-select B (Merck)

**Mobile phase:** Gradient. A was 12.5 mM NaOH in MeOH. B was 12.5 mM NaOH in water. A:B 10:90 for 5 min, to 90:10 over 15 min, maintain at 90:10 for 5 min, return to initial conditions over 1 min, re-equilibrate for 5 min.

**Flow rate:** 1

**Injection volume:** 50

**Detector:** UV 230, 254

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## CHROMATOGRAM

**Retention time:** 14.5

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## OTHER SUBSTANCES

**Extracted:** alprenolol, amitriptyline, bromazepam, carbamazepine, chlorpromazine, clonazepam, desipramine, diazepam, flunitrazepam, haloperidol, nitrendipine, nordiazepam, nortriptyline, pindolol, zolpidem

**Also analyzed:** acebutolol, acetaminophen, alprazolam, amphetamine, atenolol, betaxolol, brotizolam, caffeine, camazepam, captopril, chloroquine, clobazam, clomipramine, clothiapine, clotiazepam, clocazepam, cocaine, codeine, diclofenac, dihydralazine, dihydrocodeine, dihydroergotamine, diphenhydramine, domperidone, doxepin, droperidol, ergotamine, ethyl loflazepate, fenethylline, fluoxetine, flupentixol, flurazepam, furosemide, gliclazide, hydrochlorothiazide, hydroxyzine, ibuprofen, imipramine, ketazolam, lopraxolam, lorazepam, lormetazepam, maprotiline, medazepam, mepyramine, methadone,

methaqualone, methyl dopa, methylphenidate, metoclopramide, metoprolol, mexiletine, mianserin, midazolam, minoxidil, morphine, nadolol, nitrazepam, oxprenolol, papaverine, pentazocine, phenprocoumon, phenylbutazone, pipamperone, piritramide, practolol, prazepam, prazosin, promazine, promethazine, propoxyphene, propranolol, prothipendyl, quinine, sotalol, sulpride, thioridazine, trazodone, triazolam, trimipramine, tripeleminamine, tyramine, verapamil, yohimbine

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## REFERENCE

Lambert, W.E.; Meyer, E.; De Leenheer, A.P. Systematic toxicological analysis of basic drugs by gradient elution of an alumina-based HPLC packing material under alkaline conditions, *J. Anal. Toxicol.*, **1995**, *19*, 73-78.

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## SAMPLE

**Matrix:** blood, saliva, tissue, urine

**Sample preparation:** Homogenize (Polytron) tissue with 4 (whole brain) or 8 (brain striata) volumes of 100 mM pH 4.5  $\text{NaH}_2\text{PO}_4$  containing 0.5% NaF. Add 500  $\mu\text{L}$  brain homogenate or 500  $\mu\text{L}$  plasma, saliva, or urine containing 15  $\mu\text{L}$  saturated NaF solution to 75  $\mu\text{L}$  150  $\mu\text{g/mL}$  IS, add 50  $\mu\text{L}$  50% perchloric acid, mix vigorously for 10 s, let stand at room temperature for 10 min, add 1 mL water, mix briefly, centrifuge at  $10^\circ$  at 2500 (?) for 30 min. Remove the supernatant and add it to 750  $\mu\text{L}$  saturated sodium carbonate solution, mix briefly, add 7.5 mL pentane:chloroform 95:5, rock gently for 10 min, centrifuge in a desk-top centrifuge for 2 min, freeze in dry ice/acetone for 2 min. Remove the organic layer and add it to 250  $\mu\text{L}$  100 mM HCl, mix vigorously for 10 s, centrifuge in a desk-top centrifuge for 1-2 min, freeze in dry ice/acetone for 3-5 min, discard the organic layer. Allow the aqueous layer to thaw, remove any trace of organic solvent with a stream of nitrogen, inject a 75  $\mu\text{L}$  aliquot of the aqueous layer.

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## HPLC VARIABLES

**Guard column:** 15  $\times$  3.2  $\mu\text{m}$  Brownlee RP-8

**Column:** 250  $\times$  4.6  $\mu\text{m}$  Zorbax RX-C18

**Mobile phase:** MeCN:buffer 18:82 (Buffer was 100 mM  $\text{K}_2\text{HPO}_4$  containing 0.5% triethylamine, adjusted to pH 2.7 with phosphoric acid.)

**Flow rate:** 2

**Injection volume:** 75

**Detector:** UV 235

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## CHROMATOGRAM

**Retention time:** 8.3

**Internal standard:** 2 $\beta$ -carbomethoxy-3 $\beta$ -(4-chlorophenyl)tropane (RTI-31) (Research Biochemical International, Natick MA) (11.4)

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## OTHER SUBSTANCES

**Extracted:** clozapine, cocaine, gepirone, methylphenidate, pentazocine, pseudococaine

**Simultaneous:** acetaminophen, acetophenazine, amoxapine, amphetamine, atropine, bupropion, buspirone, caffeine, carbamazepine, chlorpheniramine, codeine, dextromethorphan, diazepam, diphenhydramine, flupenthixol, flurazepam, haloperidol, hydargine, hydrocodone, hydromorphone, lidocaine, loxapine, mepazine, meperidine, mesoridazine, methaqualone, 3,4-methylenedioxymphetamine, 3,4-methylenedioxymethylamphetamine, 3,4-methylenedioxymethamphetamine, morphine, norcocaine, oxazepam, pentobarbital, phenylpropanolamine, procainamide, procaine, propyl benzoylcegonine, quinidine, quinine, salicylic acid, secobarbital, theophylline, trazodone, 3-tropanyl-3,5-dichlorobenzoate, vancomycin, WIN 35428

**Noninterfering:** amitriptyline, benztropine methanesulfonate, butaperazine, butriptyline, carphenazine, chlorpromazine, clomipramine, cyclobenzaprine, dextropropoxyphene, dronabinol, ephedrine, ethchlorvynol, fluoxetine, fluphenazine, imipramine, meprobam-



ate, methadone, methamphetamine, nicotine, norfluoxetine, nortriptyline, PCP, phenothiazine, pseudoephedrine

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**KEY WORDS**

rat; cow; plasma; brain

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**REFERENCE**

Bonate, P.L.; Davis, C.M.; Silverman, P.B.; Swann, A. Determination of cocaine in biological matrices using reversed phase HPLC: Application to plasma and brain tissue, *J.Liq.Chromatogr.*, **1995**, *18*, 3473-3494.

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**SAMPLE**

**Matrix:** blood, urine

**Sample preparation:** Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50  $\mu$ L MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood)  $\mu$ L aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

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**HPLC VARIABLES**

**Guard column:** 20 mm long Symmetry C18

**Column:** 250  $\times$  4.6 5  $\mu$ m Symmetry C8 (Waters)

**Mobile phase:** Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A: B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

**Column temperature:** 30

**Flow rate:** 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)

**Injection volume:** 10-30

**Detector:** UV 244

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**CHROMATOGRAM**

**Retention time:** 15.223

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**KEY WORDS**

whole blood

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**REFERENCE**

Gaillard, Y.; Pépin, G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149-163.

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**SAMPLE**

**Matrix:** formulations

**Sample preparation:** Open capsules, weigh out amount equivalent to 5 mg clidinium bromide, add 15 mL water, sonicate for 10 min with gentle swirling. Make up to 25 mL with water, centrifuge, inject a 10  $\mu$ L aliquot.

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**HPLC VARIABLES**

**Column:** 250  $\times$  4.6 10  $\mu$ m Partisil 10 ODS-3

**Mobile phase:** MeCN:300 mM (NH<sub>4</sub>)H<sub>2</sub>PO<sub>4</sub> 32:68, adjust pH to 4.3  $\pm$  0.1 with 10% phosphoric acid

**Flow rate:** 1

**Injection volume:** 10

**Detector:** UV 235

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#### CHROMATOGRAM

**Retention time:** 7.1

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#### OTHER SUBSTANCES

**Simultaneous:** clidinium bromide, impurities

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#### KEY WORDS

capsules

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#### REFERENCE

Yuen, S.M.; Lehr, G. Liquid chromatographic determination of clidinium bromide and clidinium bromide-chlordiazepoxide hydrochloride combinations in capsules, *J.Assoc. Off. Anal. Chem.*, **1991**, *74*, 461-464.

---

#### SAMPLE

**Matrix:** reaction mixtures

**Sample preparation:** Irradiate MeCN solutions with UV light, inject a 20-100  $\mu$ L aliquot.

---

#### HPLC VARIABLES

**Column:** 100  $\times$  4.6 5  $\mu$ m Nucleosil C18

**Mobile phase:** MeCN:THF:60 mM pH 5.8 phosphate buffer 22:2:76

**Column temperature:** 20

**Flow rate:** 1

**Injection volume:** 20-100

**Detector:** UV 265

---

#### CHROMATOGRAM

**Retention time:** 10.25

**Limit of detection:** 0.5 ng

---

#### OTHER SUBSTANCES

**Extracted:** desmethylchlordiazepoxide, demoxepam, desmethyldiazepam, oxazepam

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#### REFERENCE

Soentjens-Werts, V.; Dubois, J.G.; Atassi, G.; Hanocq, M. High-performance liquid chromatographic determination of chlordiazepoxide, its metabolites and oxaziridines generated after UV irradiation, *J.Chromatogr.A*, **1994**, *662*, 255-262.

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#### SAMPLE

**Matrix:** solutions

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#### HPLC VARIABLES

**Guard column:** 30  $\times$  2.1 Spheri-5 RP-8

**Column:** 220  $\times$  2.1 Spheri-5 RP-8

**Mobile phase:** Gradient. A was 0.08% diethylamine and 0.09% phosphoric acid in water, pH 2.3. B was MeCN:water 90:10 containing 0.08% diethylamine and 0.09% phosphoric acid. A:B 95:% for 2 min, to 0:100 over 15 min, maintain at 0:100 for 5 min.

**Column temperature:** 50

**Flow rate:** 0.5

**Detector:** UV 200

---

#### CHROMATOGRAM

**Retention time:** 10.5

**OTHER SUBSTANCES**

**Simultaneous:** desalkylflurazepam, diazepam, flurazepam, norchlordiazepoxide, nordiazepam, oxazepam, prazepam

---

**REFERENCE**

*Rainin Catalog 1991-2*, p. 3.26.

---

**SAMPLE**

**Matrix:** solutions

**Sample preparation:** Prepare a 0.5 mg/mL solution in MeOH, inject a 5  $\mu$ L aliquot.

---

**HPLC VARIABLES**

**Column:** 250  $\times$  4.6 Zorbax RX

**Mobile phase:** Gradient. A was 150 mM phosphoric acid and 50 mM triethylamine. B was MeCN:water 80:20 containing 150 mM phosphoric acid and 50 mM triethylamine. A:B 100:0 for 2.2 min then to 0:100 over 30 min.

**Column temperature:** 30

**Flow rate:** 2

**Injection volume:** 5

**Detector:** UV 210

---

**CHROMATOGRAM**

**Retention time:** 13.1

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**OTHER SUBSTANCES**

**Simultaneous:** acetaminophen, aprobarbital, butabarbital, chloroxylenol, chlorpromazine, clenbuterol, cortisone, danazol, diflunisal, doxapram, estrone, fluoxymesterone, mefenamic acid, methyltestosterone, nicotine, oxazepam, phentermine, phenylpropanolamine, progesterone, sulfamethazine, sulfanilamide, testosterone, testosterone propionate, tralcypramine, tripeleennamine

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**KEY WORDS**

details for purification of triethylamine in paper

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**REFERENCE**

Hill,D.W.; Kind,A.J. The effects of type B silica and triethylamine on the retention of drugs in silica based reverse phase high performance chromatography, *J.Liq.Chromatogr.*, **1993**, *16*, 3941–3964.

---

**SAMPLE**

**Matrix:** solutions

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**HPLC VARIABLES**

**Guard column:** 30  $\times$  2.1 Spheri-5 RP-8

**Column:** 220  $\times$  2.1 Spheri-5 RP-8

**Mobile phase:** Gradient. A was 0.08% diethylamine and 0.09% phosphoric acid in water, pH 2.3. B was MeCN:water 90:10 containing 0.08% diethylamine and 0.09% phosphoric acid. A:B 95:5 for 2 min, to 0:100 over 15 min (?), maintain at 0:100 for 5 min.

**Column temperature:** 50

**Flow rate:** 0.5

**Detector:** UV 200

---

**CHROMATOGRAM**

**Retention time:** 10.5

---

**OTHER SUBSTANCES**

**Simultaneous:** norchlordiazepoxide, oxazepam, nordiazepam, desalkylflurazepam, diazepam, flurazepam, prazepam

**Also analyzed:** amitriptyline, amphetamine, chlorpromazine, desipramine, desmethyldoxepin, diethylpropion, doxepin, ephedrine, fenfluramine, imipramine, mesoridazine, methamphetamine, nortriptyline, phentermine, phenylpropanolamine, promazine, thioridazine, thiothixene, trifluoperazine

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## REFERENCE

*Rainin Catalog, C1-94, 1994, p. 7.24.*

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## SAMPLE

**Matrix:** solutions

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## HPLC VARIABLES

**Guard column:** 30 × 3.2 7 µm SI 100 ODS (not commercially available)

**Column:** 150 × 3.2 7 µm SI 100 ODS (not commercially available)

**Mobile phase:** MeCN:buffer 31.2:68.8 (Buffer was 6.66 g KH<sub>2</sub>PO<sub>4</sub> and 4.8 g 85% phosphoric acid in 1 L water, pH 2.3.)

**Flow rate:** 0.5-1

**Detector:** UV 240

---

## CHROMATOGRAM

**Retention time:** 1.5

**Internal standard:** 5-(4-methylphenyl)-5-phenylhydantoin (7.3)

---

## OTHER SUBSTANCES

**Also analyzed:** aspirin, carbamazepine, chlorprothixene, clonazepam, caffeine, diazepam, doxylamine, ethosuximide, furosemide, haloperidol, hydrochlorothiazide, methocarbamol, methotrimeprazine, nicotine, oxazepam, procaine, promazine, propafenone, propranolol, salicylamide, temazepam, tetracaine, thiopental, triamterene, verapamil, zolpidem, zopiclone

---

## REFERENCE

Below,E.; Burrmann,M. Application of HPLC equipment with rapid scan detection to the identification of drugs in toxicological analysis, *J.Liq.Chromatogr.*, **1994**, *17*, 4131-4144.

---

## SAMPLE

**Matrix:** solutions

**Sample preparation:** Dilute in MeOH to a concentration of 10-80 mg/mL, inject an aliquot

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## HPLC VARIABLES

**Column:** 150 × 3.9 5 µm Nova pak RP 18

**Mobile phase:** MeOH:water 50:50

**Column temperature:** 50

**Flow rate:** 0.82

**Injection volume:** 20

**Detector:** UV 254

---

## CHROMATOGRAM

**Retention time:** 13

---

## OTHER SUBSTANCES

**Simultaneous:** bromazepam, nitrazepam, flunitrazepam, clobazam, lorazepam, oxazepam, tofisopam, chlorazepate, diazepam

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## KEY WORDS

conditions are optimized

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**REFERENCE**

Guillaume,Y.; Guinchard,C. Study and optimization of column efficiency in HPLC: Comparison of two methods for separating ten benzodiazepines, *J.Liq.Chromatogr.*, **1994**, *17*, 1443-1459.

---

**SAMPLE**

**Matrix:** solutions

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**HPLC VARIABLES**

**Column:** 250 × 4.6 Zorbax RX

**Mobile phase:** Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.

**Column temperature:** 30

**Flow rate:** 2

**Detector:** UV 210

---

**OTHER SUBSTANCES**

**Also analyzed:** acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepam, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbomal, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapson, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenpropfen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarboxtyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephénytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyl dopa, methyl dopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nyldrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantone, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxazole, sulfanilamide, sulfapyridine, sulfasoxazole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline,

tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranlycypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripeleennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

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**REFERENCE**

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

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**SAMPLE**

**Matrix:** solutions

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**HPLC VARIABLES**

**Column:** 150 × 3.9 4 μm Nova pack C18

**Mobile phase:** MeOH:water 52:48

**Column temperature:** 48

**Flow rate:** 0.8

**Injection volume:** 20

**Detector:** UV 254

---

**CHROMATOGRAM**

**Retention time:** 11

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**OTHER SUBSTANCES**

**Simultaneous:** bromazepam, clobazam, clorazepate, diazepam, flunitrazepam, lorazepam, nitrazepam, oxazepam, tofisopam

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**REFERENCE**

Guillaume,Y.; Guinchard,C. Thermodynamic behavior of mixed benzodiazepines by a new liquid chromatographic method, *Chromatographia*, **1995**, *40*, 193–196.

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**SAMPLE**

**Matrix:** solutions

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**HPLC VARIABLES**

**Column:** 150 × 3.9 4 μm Nova pak C18

**Mobile phase:** MeCN:water 57:43

**Column temperature:** 44

**Flow rate:** 1.1

**Injection volume:** 20

**Detector:** UV 254

---

**CHROMATOGRAM**

**Retention time:** 10

---

**OTHER SUBSTANCES**

**Simultaneous:** bromazepam, clobazam, clorazepate, diazepam, flunitrazepam, lorazepam, nitrazepam, oxazepam, tofisopam

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**REFERENCE**

Guillaume,Y.; Guinchard,C. Marked difference between acetonitrile/water and methanol/water mobile phase systems on the thermodynamic behavior of benzodiazepines in reversed phase liquid chromatography, *Chromatographia*, **1995**, *41*, 84–87.

---

**SAMPLE**

**Matrix:** solutions

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**HPLC VARIABLES**

**Column:** 250 × 4.6 5 µm Supelcosil LC-DP (A) or 250 × 4.5 µm LiChrospher 100 RP-8 (B)

**Mobile phase:** MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)

**Flow rate:** 0.6

**Injection volume:** 25

**Detector:** UV 229

---

**CHROMATOGRAM**

**Retention time:** 6.85 (A), 5.26 (B)

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**OTHER SUBSTANCES**

**Also analyzed:** acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephénytoin, mepivacaine, mesoridazine, metaproterenol, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyl-dopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfapyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocinide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

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**KEY WORDS**

also details of plasma extraction

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**REFERENCE**

Koves, E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J. Chromatogr. A*, **1995**, 692, 103–119.

---

**SAMPLE**

**Matrix:** solutions

**Sample preparation:** Inject an aliquot of a solution in mobile phase.

---

**HPLC VARIABLES**

**Column:** Nova-Pak C18

**Mobile phase:** MeOH:buffer 85:15 (Buffer was 90.7 mL 66.7 mM Na<sub>2</sub>HPO<sub>4</sub> and 9.3 mL 66.7 mM KH<sub>2</sub>PO<sub>4</sub> made up to 1 L with water, pH 7.6.)

**Flow rate:** 5 (sic)

**Injection volume:** 20

**Detector:** UV (wavelength not given)

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#### CHROMATOGRAM

**Retention time:** 6.06

**Limit of detection:** 100 nM

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#### OTHER SUBSTANCES

**Simultaneous:** diazepam, flurazepam, nitrazepam

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#### KEY WORDS

comparison with capillary electrophoresis; capillary GC; and polarography

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#### REFERENCE

McGrath,G.; McClean,S.; O'Kane,E.; Smyth,W.F.; Tagliaro,F. Study of the capillary zone electrophoretic behaviour of selected drugs, and its comparison with other analytical techniques for their formulation assay, *J.Chromatogr.A*, **1996**, 735, 237-247.

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#### SAMPLE

**Matrix:** urine

**Sample preparation:** 2 mL Urine + 3 mL 5 M NaOH, vortex 30 s, add 12 mL diethyl ether, rotate for 5 min, centrifuge at 2500 rpm for 5 min. Remove the ether layer and evaporate it to dryness at 40° under a stream of nitrogen, reconstitute in 2 mL mobile phase, inject a 50 µL aliquot.

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#### HPLC VARIABLES

**Column:** 250 × 4.6 10 µm Alltech C18

**Mobile phase:** MeOH:water 50:50 containing 7 mL/L butylamine, adjusted to pH 3.2 with sulfuric acid

**Flow rate:** 1.8

**Injection volume:** 50

**Detector:** E, Bioanalytical Systems Model LC4B, dual glassy carbon working electrode cell half operated in the parallel mode + 1.0 V and +0.9 V, stainless steel auxiliary electrode cell half, Ag/AgCl reference electrode. The detector was preceded by a Photronix Model 816 UV irradiator which irradiated the mobile phase in a 9.144 m length of 0.5 mm i.d. × 1.6 mm o.d. Teflon tubing in a three-dimensional figure eight configuration. The irradiation apparatus was maintained at 0-5° using an ice bath.

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#### CHROMATOGRAM

**Retention time:** 6

**Limit of detection:** 2 ppb

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#### OTHER SUBSTANCES

**Simultaneous:** methylphenidate, phenobarbital, nitrazepam

**Interfering:** cocaine

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#### KEY WORDS

post-column photochemical derivatization

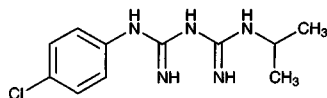
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#### REFERENCE

Selavka,C.M.; Krull,I.S.; Lurie,I.S. Photolytic derivatization for improved LCEC determinations of pharmaceuticals in biological fluids, *J.Chromatogr.Sci.*, **1985**, 23, 499-508.



# Chlorguanide



**Molecular formula:**  $C_{11}H_{16}ClN_5$

**Molecular weight:** 253.73

**CAS Registry No.:** 500-92-5, 637-32-1 (HCl)

**Merck Index:** 2138

**Lednicer No.:** 1 115

## SAMPLE

**Matrix:** blood

**Sample preparation:** 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform: isopropanol:n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100  $\mu$ L mobile phase, centrifuge at 2800 g for 5 min, inject a 50  $\mu$ L aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

## HPLC VARIABLES

**Column:** 300  $\times$  3.9 4  $\mu$ m NovaPack C18

**Mobile phase:** MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic))  $KH_2PO_4$  adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)

**Column temperature:** 30

**Flow rate:** 0.8

**Injection volume:** 50

**Detector:** UV 259

## CHROMATOGRAM

**Retention time:** 6.55

**Limit of detection:** <120 ng/mL

## KEY WORDS

whole blood; plasma; interferences may occur—compounds(all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylcegonine; acetaminophen; diazoxide; dacarbazine; sulfipyrazole; flumazenil; sulpride; morphine; atenolol; tolaxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phenacyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol;

aceprometazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluoperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opiipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tiocloamarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

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## REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

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## SAMPLE

**Matrix:** blood, urine

**Sample preparation:** Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200–350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

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## HPLC VARIABLES

**Guard column:** 20 mm long Symmetry C18

**Column:** 250 × 4.6 5 µm Symmetry C8 (Waters)

**Mobile phase:** Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A: B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

**Column temperature:** 30

**Flow rate:** 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)

**Injection volume:** 10–30

**Detector:** UV 200.5

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## CHROMATOGRAM

**Retention time:** 13.61

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## KEY WORDS

whole blood

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## REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.